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POTENTIAL OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITES BACTERIOPHORA* POINAR AS BIOCONTROL AGENT OF PINK BOLLWORM

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ABSTRACT: ***Laboratory tests indicated that in the 4th instar larvae and normal pupae of Pink bollworm (PBW) , Pectinophora gossypiella (Saunders)., the initial population (Pi) of the entomopathogenic nematode Heterorhabdites bacteriophora Poinar ranged from 4.8 to 12 and 2.7 to 13.3 when exposed to 24 and 72 h, respectively. The nematode final population numbers (Pf) were very high in the cadavers of 4th instar (ranged from 15167 to 16457 IJs / larva) followed by the wounded pupae (ranged from 2100 to 14000 IJ_S/pupa) within 14 days period. In first instar larvae, the number of Pi was 1.3 and 1.98 after 12 and 24 h, respectively. It was observed that, the penetrated nematode developed to adult stage but did not produce any progeny.***

The inoculum level of 600 IJs of H. bacteriophora caused 78, 84 and 92% mortality of 4th instar larvae and 70, 100 and 98% mortality of wounded pupae within 24, 48 and 72 h exposure period, respectively. The inoculum level of 100 IJs caused 28, 38 and 92% mortality of 4th instar larvae and 34, 70 and 82% mortality of wounded pupae within 24, 48 and 72h exposure period, respectively. The percentage mortality (M %) of normal pupae were very low in comparison with that of 4th instar larvae or wounded pupae at all inoculum levels and exposure periods. In the first instar, the highest percentage of mortality (98 %) was recorded at the inoculum level 600 IJs after 24 h while the lowest one (2%) was recorded at the inoculum level 100 IJs after 9 h.

Green house experiment indicated that, the highest M % of the 4th instar larvae (66% and 74%) were recorded at the highest inoculum level (14000 IJs /pot). The lowest M % (50% and 54%) was achieved at the lowest inoculum level (3500 IJs/pot). The highest M% was found when the insect host was placed below the soil surface at 4 cm depth. The entomopathogenic nematode H. bacteriophora revealed a significant effect against the different stages of the Pink bollworm especially the 4th instar larvae and the wounded pupae.

***Key words**: Entomopathogenic nematode, Heterorhabdites bacteriophora, Pink bollworm, Pectinophora gossypiella, Biological control.*

INTRODUCTION

The Pink bollworm, *Pectinophora gossypiella* Saunders, is one of the most destructive pests on cotton yields in Egypt. Towards the end of the cotton season, the full grown larvae (a diapauses phase and the overwintering larvae) carry over the infestation to the following crop causing more difficulty for controlling this pest (Abul-Nasr *et al*. 1974). To date, the most common method used to control the bollworms is by application of insecticides. However, pesticide resistance and residues, environmental concerns such as the impact of pest management on beneficial species, increasing pest problems associated with increasing acreage and economics. All of these issues are major driving forces in the search for new biological control agents (Gerson and smiley, 1990).Therefore, the new pest control strategies including biological control and integrated pest management (IPM) were implemented to replace or to complement chemical control methods (Wysoki, 1993).

One of the alternatives to chemical is the use of entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae. They have the ability to search for the host, are relatively safe to non-target organisms, have been exempted from registration by the Environmental protection agency, have potential to recycle in the environment, and are compatible with other control strategies. Furthermore, the Steinernematidae and Heterorhabditidae are mass-produced easily, have a wide host range, and usually killing their host within 48h (Pionar 1979 and Woodring & Kaya, 1988). In this line, cotton fields in USA were treated with the entomopathogenic nematodes, *Steinernema riobravis* for the control of *P. gossypiella*, so the resultant number of infested cotton bolls was significantly reduced and, in turn, treated plots yielded 19% higher than those of untreated plots (Gouge *et al.* 1996).

The present study aimed to evaluate the efficiency of the local isolated entomopathogenic nematode *Heterorhabditis bacteriophora* Pionar from the Egyptian soils against Pink bollworm, *P. gossypiella* under laboratory and greenhouse conditions.

MATERIALS AND METHODS

1- Isolation and identification of the Entomopathogenic Nematodes (EPNs):

Late instar larvae of the greater wax moth *Galleria mellonella* (L.) were used as a trap insect to isolate the EPNs from sandy soil samples collected from Elnobaria region according to the methods of Bedding & Akhurst (1975). The isolated nematode were identified as *Heterorhabditis bacteriophora* Poinar depending on a key proposed by Stock and Kaya (1996) based on the characters and measurements of the third stage of the infective juveniles (IJs).

2- Nematode culture:

H. bacteriophora **(Hb) was cultured on the last instar larvae of *G. mellonella*. Five larvae were placed in a petri dish (10 cm dim) lined with moistened filter paper and exposed to 250 IJs at 25 ±1°C. Two days later, dead insect larvae (cadavers) were removed, rinsed thoroughly in tap water, then transferred to White trap dishes (White, 1927). After10-12 days, the IJs were harvested from White traps and stored in distilled water at 10 °C for up two weeks prior to use (Woodring and Kaya 1988). For obtaining a surplus of nematode progeny, a new stock as suspension was made before use and the IJs were examined microscopically for viability.**

3- Insect source.

The 1st; 4th larval instars and pupal stages of PBW were obtained from mass rearing culture at Bollworms Research Dept., Plant Protection Research Institute at Doki Giza, Egypt. The greater wax larvae were obtained from a culture maintained at Plant Protection Dep. Fac. Of Agric. Ain Shams Univ., Cairo, Egypt.

4- Laboratory Bioassay Experiments:

In the present study the 1st and 4th larval instar as well as the one-day old (1-d. old) of normal or wounded pupae (pupae were punctured twice dorsally with a size 0 insect pin through outside of the integument into the haemocoel) of PBW were tested for their possible role as hosts of the Hb nematode.

In all replicates, ten individuals of each insect stage were placed in a glass Petri dish (1.5 cm deep by 10 cm diameter) containing a piece of Wattman No-1 filter paper (90 mm). The IJs were applied to the filter paper in 1 ml of water. Treated and control checks of insect stages in the Petri dishes were held in plastic bags to minimize the desiccation and then incubated at 25 ±1°C. In all experiments, treatments were replicated six times.

4.1- Effect of exposure periods of insect stages to IJs on nematode penetration and reproduction:

Six replicates from each insect stage were inoculated with 150 IJs/ replicate. The 4th larval instars and pupal stage were exposed to 24, 48 and 72 h while the 1st instars were exposed to 9, 12 and 24h. After each exposure period, dead and alive individuals for three replicates of each treatment were dissected to estimate the number of initial population (Pi) of IJs per insect stage. The other three replicates were placed in White traps and incubated for 14 days, then, the released IJs were counted per insect stage to estimate the final population (Pf) and rate of reproduction (Rr).

4.2- Effect of exposure period and the levels of nematode inoculum on the mortality percentages (M%) of insect stages of PBW:

To investigate the effect of different concentrations of the IJs of Hb by the time (the exposure period) on M% of PBW stages, six replicates of each insect stage (1st, 4th istar larvae and pupal stage) were inoculated with 100, 150, 300 and 600 IJs / replicate using the same procedure described previously, then exposed to 24 , 48 and 72h except the 1st instar larvae where they were exposed to 9, 12 and 24h. After each exposure period, M% per each inoculum level of IJs/insect stage were computed.

5- Green house Experiment:

Effect of the increasing inoculum levels of Hb on the M% of the 4th instar of PBW after 72 h at different depth:

The greenhouse experiment was conducted at 25 ± 5 °C to investigate the possible role of Hb nematode on killing the diapousing PBW larvae in soil. Seeds of cotton, *Gossypium barbadense* were sown in 30 cm pots filled with sterilized soil (sand : clay mixture 1:1). After three weeks, seedlings were thinned to three/pot. Three months later, diapousing PBW larvae were placed individually in bags (2 cm width and 4 cm length made from a plastic cloth net) and were placed at 2 and 4 cm depth below the soil surface of the pots. Each pot contained 10 bags and inoculated with 3500, 7000 or 14000 IJs of Hb/each depth.

Each treatment was replicated three times and uninoculated pots were kept as a check. Watering was done when needed. After 72 h from nematode addition, caged larvae were recovered and the numbers of living and dead insects were recorded/pot. The dead insects were dissected to assure nematode infection and, M% were calculated.

Statistical analysis

The obtained data were analyzed by using a Two Way ANOVA completely randomized with Duncan's multiple range tests using co-stat program computer.

RESULTS AND DISCUSSION

1- Effect of exposure period and insect stage on nematode penetration and reproduction:

In the present study data given in (Table 1) indicated that, the EPNs *H. bacteriophora* was differed in its virulence, penetration and reproduction in PBW based on the exposure period and the insect stage.

It was found that, there were positive correlations between the number of nematode harbored the infected I,,,,nsects and the length of exposure time. In the 4th instar larvae and normal pupae, the initial population (Pi) ranged from 4.8 to 12 and 2.7 to 13.3 when exposed to 24 and 72 h respectively.

Regarding to the wounded pupae, the Pi was independent of exposure time. Moreover the Pi was higher than that of the other normal pupae.

As for the rate of reproduction (Rr), results indicated that the Rr was high after 24h for the different insect stages, and sharply decreased after 48 and 72hrs.

Similar results obtained by Shannag *et al* (1994) who recorded that, larval mortality and penetration rate of IJs into the cucurbit pest *Diaphania nitidalis* were positively correlated to host exposure time for *S. carpocapsae* ; *S. feltiae* and *H. bacteriophora* .

As indicated in Table (1), the final population of nematode (Pf) was very high in the 4th instar (ranged from 15167 to 16457 IJs / 1 larva) whereas the wounded pupae was comparatively lower (ranged from 2100 to 14000 IJ_S/1pupa) after 14 days period. Furthermore, it was observed that the increasing number of penetrating IJs was inversely related to the produced IJs progeny. Similar observation was demonstrated by El-sayed, (2002). This means that when low numbers of IJs penetrated the host, nematode reproduction continues while food resources in the insect cadaver are depleted, allowing for two to three generations. Conversely, if the food supply is depleted by the large number of the penetrated IJs, the first-generation develop directly into lower numbers of IJs.

Table (1): Effect of the exposure period of the 4th instar larvae and pupal stages of PBW to IJs of Hb on the penetration (Pi), final population (Pf) and rate of reproduction (Rr).

Exposure Period (h)	Pi, Pf and Rr of IJs within insect stage								
	Larval stage			Pupal stage					
	4th instar			Normal pupae			wounded pupae		
	Pi	Pf	Rr	Pi	Pf	Rr	Pi	Pf	Rr
24h	4.8c	16457a	3428.5	2.7b	4888b	1810.4	11.7a	9718a	830.6
48h	8.4b	15565b	1853.0	4.0b	4168b	1042.0	10.0a	14000a	1400.0
72h	12a	15167b	1264.0	13.3a	7387a	555.4	15a	2100b	140.0
LSD .05	2.81			1.88			5.91		

Means with the same litter within each column are not significantly different.
IJs: Infective juveniles
Pi: Initial population (Mean No. of IJs Penetrated in insect stage).
Pf: Final population (Mean No. of IJs reproduced within each insect cadaver after 14 days of incubation).
Rr: Rate of reproduction (= Pf/Pi).

With respect of the first instar of PBW and the lethal effects of Hb, the nematode initial populations (Pi) were increased with increasing of the exposure periods (Table, 2). In addition, it was noticed that, the penetrated nematode developed to adult stage but did not produce any IJs (Pf = 0). This phenomenon may be attributed to the small size of the neonate larvae (Shannag, *et al.* 1994).

Table (2): Effect of the exposure period of the1st instar larvae of PBW to IJs of Hb on the penetration (Pi), final population (Pf) and rate of reproduction (Rr).

Exposure Period (h)	Pi, Pf and Rr of IJs within 1st instar		
	Pi	Pf	Rr
9h	0.0b	0.0	0.0
12h	1.3ab	0.0	0.0
24h	1.98a	0.0	0.0
LSD .05	1.48		

Means with the same litter within each column are not significantly different.
IJs: Infective juveniles
Pi: Initial population (Mean No. of IJs Penetrated in insect stage).
Pf: Final population (Mean No. of IJs reproduced within each insect cadaver).
Rr: Rate of reproduction (= Pf/Pi).

2- Effect of exposure periods and nematode inoculum levels on the M% of larval and pupal stages of PBW:

Data in Table (3) revealed that, M% of normal pupae were very low in comparison with that of 4th instar larvae or wounded pupae at all inoculum levels and exposure periods. This may be attributed to the hard integument of pupae which reduce nematode penetration. Conversely, the wounded pupae and the soft integument of larval became more susceptible to nematode infection. Similar results were obtained by Lindegren *et al.,* (1993) who stated that, the pupae of PBW were not susceptible to nematode infection unless they were injured. Generally, it could be concluded that, increasing IJs levels from 100 to 600 IJs caused an increase in nematode infectivity, which led to rapid death of the host. Similarly, increase exposure period from 24 to 72h of each inoculum level increase the M %. Results are in agreement with the finding of Shannag *et al.* (1994) who found that, mortality caused by *H. bacteriophora* and *S. feltiae* increased progressively 27 and 20% at 1 h to 93 and 76% after exposure period of 24 h, respectively. Nevertheless, the present results indicated that, equivalent rates of M % of all insect stages were obtained after 72 h exposure period mostly at all inoculum levels. Seemingly, prolong exposure time gave a chance to the

symbiotic bacteria for more propagation which led to the increase of the M% even at low inoculum levels.

Table (3): Effect of different inoculum levels of IJs of Hb on the M% of 4th instar larvae and pupal stages of PBW after 24h, 48h and 72h.

Nematode inoculum/ insect stage	M (%) of insect stages								
	Larval stage			Pupal stage					
	4th instar			Normal pupa			wounded pupa		
	24 h	48h	72h	24 h	48h	72h	24 h	48h	72h
100	28a	38c	92a	14b	18b	30b	34b	70b	82b
150	38b	60b	78b	18b	28b	38b	38b	78b	90ab
300	72a	82a	90a	28ab	36ab	58a	64a	96a	96a
600	78a	84a	92a	44a	52a	66a	70a	100a	98a
LSD .05	1.22	1.19	0.85	1.77	1.94	1.53	2.58	1.25	0.87

Means with the same litter within each column are not significantly different.

Regarding to 1st instar larvae data in Table (4) indicated that, the M % followed the same trend as well as with the 4th instar larvae. By comparison, it seems that the first instar (Table 4) were less affected than those of the fourth instar and pupae (Table 3). This may be due to the small size of the neonate larvae which are less attractive to the IJs (Shannag *et al.,*1994).

Table (4): Effect of different inoculum levels of the Hb on the M% of the 1st instar of PBW after 9h, 12h and 24h.

Nematode inoculum/ 1st instar larva	M% of 1st instar		
	9 h	12h	24 h
100	2b	8c	34b
150	14b	28b	40b
300	66a	82a	90a
600	66a	86a	98a
LSD.05	1.43	1.48	2.07

Means with the same litter within each column are not significantly different.

3- Effect of the increasing inoculum levels of Hb on the M% of the 4th instar of PBW after 72 h at different depths under green house conditions.

Obtained data in Table (5) revealed that, the highest M % of the 4th instar larvae, (66% and 74%) were recorded at the higher inoculum level (14000 IJs /pot). On the other hand, the lowest M % (50% and 54%) were recorded at the lowest inoculum level (3500 IJs/pot). These results are supported by Lindegren *et al* (1992) who mentioned that, spring and fall applications of *S. carpocapsae* against PBW resulted in larval mortalities ranging from 12.9% at 1.5 nematodes per cm^2 to 100% at 50 nematodes per cm^2. Yeh and Alm (1992) added that, the larval insect mortalities were generally proportional with the rates of nematode application. Data in Table (5) also indicated that, a higher M% was found when the insect host were placed below the soil surface at 4 cm depth; on the opposite the lower M% was found at 2 cm depth. This may be attributed to the escape of EPNs from soil surface to avoid the drought. In this respect, Kaya, *et al.* (1993) found that the majority of IJs of Hb occurs deeper in the soil profile. This information is of a great value as a guide to which EPNs are likely to applied in circumstances where PBW larval harbor the soil.

Table (5) : Effect of the increasing inoculum levels of Hb nematode on the M % of the 4th instar of PBW after 72 h at two depths in soil under green house condition.

Nematode inoculum/ pot	M% of 4th instar after 72h	
	Depth	
	2 cm	4 cm
3500	50 a	54 a
7000	47 a	67 a
14000	66 a	74 a
LSD.05	22. 0876	23. 0697

Means with the same litter within each column are not significantly different.

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كفاءة النيماتودا الممرضة للحشرات هتيرورابديتيس بكتريوفورا *Heterorhabditis bacteriophora* Poinar كعامل حيوى لمكافحة دودة اللوز القرنفلية.

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الملخص العربى

- تم عزل النيماتودا المتطفله على الحشرات من تربه رمليه بمنطقة النوبارية بأستخدام يرقات العمر الأخير لدودة الشمع الكبيرة كمصيده لهذه النيماتودا. و تم تعريف النوع *Heterorhabditis bacteriophora* Poinar وهو يتبع فصيلة Family Heterorhabditidae.
- تم اكثار هذا النوع من النيماتودا معمليا على العمر اليرقى الأخير لدوده الشمع وذلك للحصول على الطور اليرقى المعدى لهذه النيماتودا وذلك لتفهم كفائته فى الاختراق والتكاثر داخل الاطوار المختلفه لدوده اللوز القرنفليه كما تم تقدير نسب الموت المختلفه عند تعريض الاطوار المختلفه للحشره الى مستويات مختلفه من العدوى بالنيماتودا وكذلك بعد فترات مختلفه من التعريض ، كما تم تقدير نسب الموت الناتجة عن العدوى بالنيماتودا للعمر اليرقى الرابع لدودة اللوز تحت ظروف الصوبة.

أولا : الاختبارات المعمليه .

أ- عند تعريض يرقات العمر الاول والرابع وطور العذراء عمر يوم واحد (عذراء لها جليد طبيعى واخرى تم عمل خدش ميكانيكى لها بدبوس (رقم صفر) المستخدم فى تثبيت الحشرات) وذلك لتركيز ١٥٠ طور معدى وفتره تعريض ٢٤ ، ٤٨ ، ٧٢ ساعه تم الحصول على النتائج التاليه :

١- بزياده فتره التعريض يزداد عدد غزو النيماتودا المخترقة حيث تراوح عددها من ٤.٨ - ١٢ يرقة نيماتودا لكل يرقة حشرية عمر رابع بينما كان ٢.٧ - ١٣.٣ يرقة نيماتودا لكل عذراء ذات الجليدالطبيعى وذلك بعد فترة تعريض من ٢٤-٧٢ ساعة على التوالى.

٢- معدل غزو النيماتودا للعذراء ذات الجليد الطبيعى اقل من نظيره فى العذراء المخدوشه ميكانيكيا .

٣- التعداد النهائى (Pf) الناتج للنيماتودا كان مرتفعا فى يرقات العمر الرابع حيث تراوح من ١٥١٦٧-١٦٤٥٧ طور معدى/ يرقة)، يلية الناتج من العذراء المخدوشة ميكانيكيا حيث تراوح من (٢١٠٠- ١٤٠٠٠ طور معدي/ عذراء).

٤- عند أختراق الطور المعدى للنيماتودا العمر اليرقى الأول للحشرة حدث له تطور حتى وصل إلى الطور الكامل ولكن لم ينتج عنه أى ذرية ، وتراوح عدد النيماتودا المخترقة من ١.٣ - ١.٩٨ بعد ١٢ ، ٢٤ ساعة على التوالى.

ب- عند دراسه تاثير التركيزات المختلفه على نسبة الموت لاطوار الحشره السابق ذكرها فقد كانت أهم النتائج هى :

١- عند زياده التركيز أو زيادة فترة التعريض داخل التركيز الواحد تزداد نسبة الموت ٧٨، ٨٤ ، ٩٢% ليرقات العمر الرابع و ٧٠ ، ١٠٠ ، ٩٨ % للعذراء المخدوشة ميكانيكيا وذلك بعد ٢٤ ، ٤٨ ، ٧٢ ساعة على التوالى.

٢- نسبه الموت فى العذراء الطبيعيه كانت اقل إذا ماقورنت بمثيلتها فى العذراء المخدوشه ميكانيكيا أو يرقات العمر الرابع.

٣- أعلى نسبة موت فى العمر الأول كانت ٩٨% عند تركيز ٦٠٠ طور معدى بعد ٢٤ ساعة بينما أقل نسبة كانت ٢% عند تركيز ١٠٠ طور معدى بعد ٩ ساعات.

ثانيا: تقدير نسبة الموت تحت ظروف الصوبة:

تم دراسة تأثير التركيزات المختلفة للنيماتودا هتيرورابديتيس بكتريوفورا على يرقات العمر الرابع لدودة اللوز القرنفلية وذلك عند وضعها على أعماق مختلفة من سطح التربة. وكان من أهم النتائج المتحصل عليها مايلى:

١- كانت أعلى نسبة موت فى يرقات العمر الرابع تتراوح من ٦٦- ٧٤% عند معدل عدوى بالنيماتودا ١٤٠٠٠ طور معدى/ أصيص.

٢- كانت أقل نسبة موت فى يرقات العمر الرابع تتراوح من ٥٠- ٥٤% عند معدل عدوى بالنيماتودا ٣٥٠٠ طور معدى/ أصيص.

٣- سجلت أعلى نسبة موت لليرقات الموضوعه تحت سطح التربة على عمق ٤ سم وذلك بالمقارنة بعمق ٢ سم.

وعموما قد أظهرت الدراسة أن النيماتودا الممرضة للحشرات من النوع *Heterorhabditis bacteriophora* Poinar لها تأثير معنوى فى نسبة الموت ضد الأطوار المختلفة لدودة اللوز القرنفلية خاصة يرقات العمر الرابع وكذلك العذراء المخدوشة ميكانيكيا مما قد يجعلها أحد العوامل الحيوية التى قد تؤخذ فى الأعتبار عند مكافحة هذه الآفة.